## REMARKS

Claims 1-48 are now pending in the application, of which Claims 23-26, and 37-44 have been previously withdrawn. Claims 1-22, 27-36 and 45-48 presently stand rejected. Minor amendments have been made to the claims to simply overcome the objections to the specification and rejections of the claims under 35 U.S.C. § 112. The Examiner is respectfully requested to reconsider and withdraw the rejection(s) in view of the amendments and remarks contained herein.

### A. Amendments to the Claims

Claims 1 and 48 are amended without prejudice to remove the words "immunogenic fragments thereof".

Claim 48 has further been amended to recite "an immunologically sufficient amount." Support for this claim amendment can be found on pages 11-12 of the specification.

## RESPONSE TO SUPPLEMENTAL FINAL OFFICE ACTION DATED MARCH 16, 2007

### REJECTION UNDER 35 U.S.C. § 112

ITEM 5. Enablement

Claims 1-22, 27-36 and 45-48 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement thereon. This rejection is respectfully traversed, and or rendered moot.

Applicants respectfully disagrees with the Examiner's finding that the specification fails to describe immunoepitopes or fragments that would be protective.

More particulary, the Examiner asserts that the specification is "silent as to what specific 'immunoepitope' or 'immunogenic fragments' confers said a given immune response." (Page 6 of the Office Action, emphasis in original). In support of the present rejection, the Examiner cites two references, the first (Bowie et al.) discusses the difficulty of predicting the function of a protein structure when the amino acids of the sequence of the protein are substituted. The other reference (Greenspan et al.) discusses the relationship between epitope structure and antibody binding. The Examiner concludes, that the "immunoepitopes that can elicit a protective immune response to a given pathogen can only be identified empirically. This constitutes undue experimentation." (Page 7 of the Office Action)

Fragments of SEQ ID NO:2, 4, or 8 can be produced using conventional molecular biological techniques. They can also be tested in vivo to determine whether they can elicit an immunological response by measuring the challenged animal's humoral or cellular response to the immunological challenge. It is well known in the art how to experimentally challenge animals with a polypeptide or fragments of proteins, obtain blood samples, and test for antigen/epitope specific antibodies, for example through the use of Enzyme Linked Immuno Sorbent Assays (ELISA).

One of ordinary skill in the art could, by routine experimentation, follow the methods set forth in the specification (Examples V-VII) to determine whether the fragments of SEQ ID NO:2, 4, or 8 were immunogenic. Such methods, even if complex, are typically engaged in by those skilled in the art.

Applicants respectfully submit that the testing of fragments of SEQ ID NO: 2, 4, or 8 to determine whether the fragments were immunogenic was routine in this art as of

the date of filing of the present application. Applicants respectfully submit that the present written description is sufficient to support a claim to an oral vaccine comprising, in an orally suitable formulation, at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the group consisting of isolated recombinant adhesion proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8, and immunogenic fragments thereof, wherein said vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.

Notwithstanding, in the interest of advancing prosecution of the present application by reducing the number of issues outstanding, Applicants has amended the claims by removing "immunogenic fragments." Such an amendment renders the present objection moot. Applicants however, reserve the right to reintroduce claims reciting "the immunogenic fragments" of SEQ ID NO:2, 4, or 8 in a continuing application, while pursuing claims as amended herein.

ITEM 7. The rejection under 35 U.S.C. 112 second paragraph is maintained for Claim 48 for failing to particularly point out and distinctly claim the subject matter. Specifically, the Examiner alleges that the terms "predetermined amount" and "predetermined volume " are unclear. Applicants respectfully traverse this rejection.

Applicants have amended Claim 48 by amending the term "predetermined amount" to "immunologically sufficient amount". Support for this amendment can be found for example, on page 12 of the specification. The Examiner has withdrawn her

rejection the term "immunologically sufficient amount" under section 112, 2nd paragraph.

Applicants respectfully assert that "predetermined volume" can be understood by an ordinary person skilled in the art as meaning a volume of water (and/or saline) and organic oil, that would result in an emulsion suitable for oral administration of the vaccine which can be determined through routine trials. Examples of predetermined volumes of organic oil can vary depending on the size of the vaccine innoculum required. The specification teaches that the volumetric ratio of the oil and water may be in the ratio of 2:1 or 1:1. Example V also teaches that excellent results were obtained when the volumes of the water (and/or saline) and organic oil are .25 ml and .5 ml, respectively. Hence, one of ordinary skill in the art can determine rather easily the predetermined volume of organic oil used to make the emulsion if the ratio of oil to water can be 2:1 or 1:1. Therefore, for a 4 mL emulsion, the predetermined volume can be 2.67mL of oil and 1.33 of water or 2 mL of oil mixed with 2 mL of water.

Applicants believe that these amendments and remarks overcome the rejection and respectfully request that it be withdrawn.

**REJECTION UNDER 35 U.S.C. § 102**

ITEM 6. Claims 1-3, 5-6, 10, 27-29 and 48 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Fang et al. (J. Fish Diseases, 2000, 23:137-145). This rejection is respectfully traversed.

Fang et al. disclose intraperitoneal immunization of blue gourami with an *Aeromonas hydrophila* (*A. hydrophila*) 43 kDa. major adhesion protein containing fraction in Freund's Complete Adjuvant (FCA).

The Examiner asserts that the preamble of Claims 1-3, 5-6, 10, 27-29 and 48 reciting "oral" is a claim limitation of "intended use." The Examiner argues that "[i]f the prior art structure is capable of performing the intended use, then it meets the claim." (page 9 of the Office Action). The Applicants respectfully disagree.

The Applicants point out that Fang et al. do not teach the use of a recombinant protein major adhesion protein and/or immobilization antigen repeat I of *Ichthyophthirius multifiliis* fusion protein that are immunogenic (effecting immunization) and **orally suitable**.

The Applicants respectfully submit that Fang et al. fail to disclose: a) any recombinant AHMA polypeptide that could effect immunization against *Aeromonas hydrophila* that would be orally suitable, or b) that Fang et al.'s major adhesion protein vaccine would effectively function as a vaccine to protect against *A. hydrophila* if it were administered orally.

The Examiner has alleged that the "[b]urden is on the Applicants to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the vaccine of the prior art does not possess the same material structural and functional characteristics of the claimed vaccine)." Citing In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and In re Fitzgerald et al., 2005 USPQ 594. (Office Action, page 8).

Applicants have provided numerous reasons why the prior art vaccine of Fang et al., does not possess the same **structural** material and **functional characteristics** of the

claimed vaccine. These differences are readily observable when the vaccines are compared in their entireties rather than comparing the AHMA protein from the two vaccines in isolation from the rest of the vaccine components. More particularly:

1). The Fang vaccine does not possess a pure recombinant AHMA protein in near-native form as set forth in any one of SEQ ID NOs: 2, 4, or 8;

2). The Fang et al vaccine does not possess an orally suitable formulation; and

3) The Fang et al vaccine cannot be functionally delivered orally.

**1. Pure recombinant AHMA Protein in Near-Native Form**

Fang et al. do not disclose or teach an oral vaccine capable of effecting immunization of an animal comprising a pure recombinant polypeptide (AHMA) having amino acid sequences selected from the group consisting of SEQ ID NOS: 2, 4, or 8, but rather a sample containing a 43 kDa. major adhesion protein concentrated from peak 1 which was not reported to be homogenous. Furthermore, the Fang et al. 43 kDa. major adhesion protein is structurally and immunologically very different to the pure recombinant AHMA presently described in Claims 1 and 48, because the 43 kDa. major adhesion protein described in Fang et al. was extracted from a crude extract using potassium isothiocyanate, a very powerful protein denaturant which is known to disrupt and denature the secondary and tertiary structure of proteins (see Fang et al. page 138. The manuscript is attached as a PDF copy with this response). In contrast, the pure recombinant AHMA is purified as a homogenous pure polypeptide using an expression system that expresses the recombinant proteins in a near-native conformation which is structurally and immunologically distinct to the 43 kDa. major adhesion protein described in Fang et al.

(see specification page 8, lines 10-11). As such, Fang et al. do not disclose or teach a recombinant, near-native, isolated AHMA protein capable of effecting immunization of an animal against *A. hydrophila* when administered orally and thus does not anticipate Claims 1 and 48 of the present application.

**2. Orally Suitable Formulation**

The claim preamble provides a positive claim limitation that when viewed in context of the entire specification, would lead a person of ordinary skill to view the antigenic components as being those that are orally suitable, and thus effecting immunization when orally administered. For example, the specification states: "These selected bacteria may be inactivated by any method known to those skilled in the art....Their antigenic proteins may also be made by recombinant methods for incorporation into the multicomponent oral vaccine" (Specification, page 9, lines 23-27).

Hence, the term "orally suitable" is not one of intended use but a positive limitation reciting vaccine components that are *orally suitable* when administered orally and that are capable of effecting immunization of an animal against *A. hydrophila*. Fang et al. neither disclose nor teach that the AHMA 43kDa. protein containing concentrate in the presence of Freud's Complete Adjuvant (FCA) would be suitable as an oral vaccine, suitable for oral administration, or capable of effecting immunization against *A. hydrophila* when administered orally. Accordingly, the Applicants submit that the preamble of the amended claims reciting an "orally suitable formulation" is not one of intended use, but a structural limitation on vaccine components that are orally suitable and that are capable of effecting immunization against *A. hydrophila* and other pathogens when orally administered.

As such, Fang et al. do not disclose or teach a recombinant, near-native, isolated AHMA protein capable of effecting immunization of an animal against *A. hydrophila* when administered orally, and thus do not anticipate Claims 1 and 48 of the present application.

**3. <u>The Fang et al vaccine cannot be functionally delivered orally.</u>**

The intraperitoneal vaccine composition in Fang et al. comprises a 43 kDa. major adhesion concentrate derived from a crude *A. hydrophila* PPD 134/91 extract and FCA (see for example pages 138-139 attached as a copy herein). The Fang et al. vaccine composition is generally not accepted as being "capable of performing the intended use" (i.e. effecting immunization of an animal against *A. hydrophila* when administered orally) most importantly because FCA is contraindicated for oral use and is considered toxic when administered orally. The oral suitability requirement of the vaccine composition of the present invention is a limitation that limits the composition of the vaccine and, as repeated throughout prosecution, is not a "method of vaccine delivery" (Office Action at page 9). An orally suitable vaccine formulation can be administered intraperitoneally, intramuscularly, intravenously, intradermally, nasally, orally or by any other administration route and thus such claim language should not be interpreted to be "method of vaccine delivery" as argued by the Examiner.

Moreover, the Examiner's own statements for patentability include, for example: 1) "It should be remembered that a recitation of the intended use of the claimed invention must result in a structural difference between the claimed invention and the prior art in order to patentably distinguish the claimed invention form the prior art", and 2) "If the prior art is capable of performing the intended use, then it meets the claim." (Office Action at

page 9). With respect to point number 1), the Applicants respectfully submit, that the intended use of the present invention, (i.e. oral administration), requires a structural difference in the composition when compared to the vaccine composition of Fang et al. which is not orally suitable because the vaccine in Fang et al. is administered intraperitoneally. With respect to point number 2), the prior art vaccine of Fang et al., is not "capable of performing the intended use" of the present invention, i.e. to be delivered orally. Thus, it is respectfully submitted that the Applicants have met the Examiner's criteria for patentably distinguishing the rejected Claims of the present Office Action over the cited prior art.

Applicants further support their position for patentability over Fang et al. because Fang et al, do not teach or suggest an "orally suitable vaccine", or an oral vaccine that can protect the subject from *Aeromonas hydrophila* infection. Fang et al., enhance immunogenicity of their antigen preparation through the use of adjuvants, in particular, a very toxic adjuvant called Freund's Complete Adjuvant. As cited in "Guidelines for the Production of Polyclonal and Monoclonal Antibodies In Rodents and Rabbits", SUNY Upstate Medical University, Committee for the Humane Use of Animals Revised 10/17/01 at http://www.upstate.edu/scripts/php_include/pdfconvert.php?file=/dlar/chua/antibodyproduction.pdf&convert=1:

> "Monoclonal and polyclonal antibodies are important reagents utilized in a variety of experimental techniques in almost every scientific discipline. Additionally, the application of monoclonal antibodies to clinical use in both diagnostic and treatment arenas is increasing. *In vitro* methods have been successfully developed for large scale monoclonal antibody production, however, animals continue to be an important source of monoclonal and polyclonal antibodies,

especially in the research setting. The purpose of these guidelines is to provide methods with demonstrated success that also minimize pain and distress for the laboratory animals employed in these techniques. Adjuvants Immunogens (substances to which antibodies are desired) are rarely sufficiently antigenic to directly induce a satisfactory immune response, therefore, they are usually administered in conjunction with adjuvants to enhance the inflammatory response at the site of administration. Historically, the single most commonly used adjuvant in the research setting has been Freund's adjuvant. Freund's adjuvant is a water-in-oil emulsion consisting primarily of mineral oil. The resultant emulsion is very viscous and can be difficult to inject. Freund's adjuvants are available in two forms: complete which contains killed *Mycobacterium tuberculosis,* or incomplete without the additional bacterial component. The oil acts as a repository which releases the immunogen over time. The mycobacterial cell wall is a potent immune enhancer. Freund's Complete Adjuvant (FCA) is extremely inflammatory and may only be used once. **Anaphylactic reactions can occur when FCA is used more than once and these reactions may be fatal**. Only Incomplete Freund's Adjuvant should be used for booster immunizations. **FCA is known to commonly produce undesirable side effects. Granuloma formation, tissue necrosis and sloughing, abscessation and fever are routinely seen. Other deleterious systemic effects, such as polyarteritis, have been reported**. FCA is considered a human biohazard since accidental self-inoculation or splashing in the eye have been shown to cause painful lesions not easily treated, as well as sensitization to tuberculin which negates future skin testing."

(emphasis added)

Thus, Fang et al., fail to teach or suggest an oral vaccine comprising, in an orally suitable formulation, at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the group consisting of isolated recombinant adhesion proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8 wherein said vaccine is capable, by oral administration in an immunologically

sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.

Accordingly, since Fang et al. do not disclose each and every limitation found in Claims 1 and 48 and Claims dependent thereon, it is respectfully submitted that Fang et al. do not anticipate the presently rejected Claims 1, and 48 and dependent claims thereon. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

**REJECTION UNDER 35 U.S.C. § 103**

ITEM 8. The rejection under 35 U.S.C. 103(a) is maintained for claims 1-6, 10, 27-29, 35-36 and 48 the reasons set forth on pages 11-13, paragraph 10 of the previous Office Action.

Fang et al. is discussed above under Item 6, above. Chen et al. disclose pharmaceutical oil-in-water emulsions for delivery of polyfunctional active ingredients. Chen et al. teach that the oil component of the oil-in-water emulsion may not be appropriately polar to effectively incorporate polyfunctional active ingredients at desirable therapeutic levels, without compromising product safety (see, for example, column 2, lines 7-12). In order to overcome this problem, Chen et al. disclose oil-in-water emulsions "wherein the oil phase includes components chosen to increase the polarity of the oil phase" (see column 3, lines 55-62). Chen et al. do not teach that the addition of an organic oil, for example palm oil, could be used to provide improved delivery of polyfunctional active ingredients. Prior to Chen et al., others in the field were well versed with emulsions containing organic oils such as palm oil for delivery of active ingredients. Chen et al. teach that addition of polarity modifiers to such an

emulsion can be directly attributed to increasing the polar nature of the oil phase which is said to improve delivery of polyfunctional active ingredients. Hence, one of ordinary skill would not combine the teachings of Fang et al. with the addition of palm oil as described in Chen et al. without the addition of polarity modifiers.

Applicants respectfully submit that there is a lack of suggestion or motivation to combine the Fang et al. reference with the Chen et al. reference because Fang et al. rely on a vaccine composition that is a water-in-oil emulsion, largely due to the addition of FCA, whereas Chen et al. describe oil-in-water emulsions for their polyfunctional active ingredient delivery (see col. 9, lines 17-19). These two emulsion systems are incompatible and thus, one of ordinary skill in the art interested in using a vaccine composition as described by Fang et al. would not be motivated to replace FCA with palm oil, or to add palm oil in addition to FCA, because in either case doing so would have been thought to decrease the immunostimulatory effect of the FCA and ultimately the effectiveness of the vaccine.

Moreover, there is no reasonable expectation of success by one of ordinary skill in the art to provide an orally suitable vaccine by substituting the paraffin oil found in Fang et al. vaccine composition with the palm oil described in Chen et al. because the deficiencies (i.e. toxicity of FCA) pointed out in the discussion of Fang et al. in Item 6 above would not be obviated by such a replacement. A vaccine having a water-in-oil emulsion comprising the components found in the Fang et al. vaccine with palm oil from Chen et al., would still render the vaccine not orally suitable, and certainly not capable of effecting immunization against *A. hydrophila* when administered orally, due to the toxicity of such a vaccine.

Accordingly, since Fang et al., either alone or in combination with Chen et al., do not disclose each and every limitation found in Claims 1 and 48 and Claims 2-6, 10, 27-29, 35-36 which are dependent thereon, it is respectfully submitted that Fang et al. in combination with Chen et al. do not render the rejected Claims 1-6, 10, 27-29, 35-36 and 48 obvious to one of ordinary skill in the art. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

ITEM 9. Claims 7-9 stand rejected under 35 U.S.C. § 103(a) as allegedly anticipated by Fang et al. in view of Chen et al. (U.S. Patent No. 6,720,001 B1, published April 13, 2004) as applied to Claims 1-6, 27-29, 35-36, and 48 and further in view of Calanchi et al. (U.S. Patent No. 5,008,117, published April 16, 1991). This rejection is respectfully traversed.

Claims 7-9 are drawn to the oral vaccine of Claim 2 further mixed with a binding agent, in particular carboxymethylcellulose. Fang et al. and Chen et al. are discussed above under Items 6 and 8. Calanchi et al. teach a method of dispersing thickening agents in pharmaceutical formulations for effective delivery of micro-encapsulated drugs, which otherwise have the tendency to precipitate or float. As discussed in Item 10 above, it would not be *prima facie* obvious to combine Fang at al. and Chen et al. Therefore, it would also not be *prima facie* obvious to add carboxymethylcellulose as taught by Calanchi et al. to the vaccine composition of Fang et al. and Chen et al.

Moreover, Calanchi et al. teach the use of carboxymethylcellulose as the thickening or suspending agent and <u>not as the binding agent</u> (see Claim 14 of Calanchi et al.). Exemplary binding agents described in Calanchi et al. also do not include

carboxymethylcellulose (see column 3, lines 63- 68 and Claim 14 of Calanchi et al.). Accordingly, Calanchi et al. teach away from the present invention. Yet the combination of Calanchi et al. to Fang et al. and Chen et al. would not provide what is lacking from the combination of Fang et al. and Chen et al.

Accordingly, since Fang et al. either alone or in combination with Chen et al. and further in view of Calanchi et al. do not disclose each and every limitation found in Claims 1 and 48 and Claims which are dependent thereon, it is respectfully submitted that Fang et al. in combination with Chen et al. and further in view of Calanchi et al. do not render the rejected Claims 7-9 obvious to one of ordinary skill in the art. Applicants believe that these remarks overcome the rejection and thus respectfully request that it be withdrawn.

ITEM 10. Claims 1-3, 5-6, 10, 15-16, 20-21, 27-36, 45, and 48 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et al. (U.S. Pat. No. 5,284,653, published February 8, 1994) in view of Fang et al. (*Journal of Fish Diseases*, 2000, 23, 137-145). This rejection is respectfully traversed.

Claims 1-3, 5-6, 10, 15-16, 20-21, 27-36, 45, and 48 are drawn to an oral vaccine comprising at least one recombinant protein AHMA and recombinant protein AHMA fragments, optionally in combination with another membrane protein (immobilization antigen repeat I of *Ichthyophthirius* multifiliis) and/or inactivated bacterial strains and/or inactivated viral strains.

As discussed above under Item 6, Fang et al. neither disclose nor teach an oral vaccine comprising, in an orally suitable formulation, at least one of isolated recombinant adhesin protein of *Aeromonas hydrophila* (AHMA) selected from the

group consisting of isolated recombinant adhesion proteins having the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 4, or 8, wherein the vaccine is capable, by oral administration in an immunologically sufficient amount, of effecting immunization of an animal against *Aeromonas hydrophila*.

Wolf-Watz et al. disclose a fish vaccine comprising a live, whole cell, avirulent, invasive and immunogenic strain of a fish pathogenic bacterial species such as *A. hydrophila.* As discussed in Wolf-Watz et al., live vaccines are capable of provoking a stronger immune reaction than killed pathogens (see col. 2, lines 26-31 and col. 3, lines 63-67) while the use of whole cells instead of a single antigenic component enables the raising of an immune response against all the surface-presented antigens of the cell thereby conferring effective immunity against the whole organism (col. 2, lines 26-32).

The Supplemental Office Action dated 3/16/2007 fails to address that Fang et al. could not be combined with Wolf-Watz et al. to arrive at the present invention because Fang et al. fails to teach the protein sequence of the adhesin protein of *Aeromonas hydrophila* (AHMA) and, therefore, is not capable of being combined with Wolf-Watz to arrive at the "vaccines comprising bacteria the [sic] carry multiple determinants from different pathogenic fish and capable of expressing hybrid (fusion) determinants (column 7)." (Office Action, page 15). As noted above, it is the Applicants and not Fang et al., that provide the recombinant amino acid sequences of the adhesin protein of *Aeromonas hydrophila* (AHMA) set forth in SEQ ID NOs 2, 4, or 8.

Furthermore, Wolf-Watz et al. unlike Fang et al., teach away from injecting antigenic determinants via parenteral routes including: intramuscular, intravenous or

intraperitoneal injections (see col. 8, lines 29-39, "...immunization by injection, although efficient in experiments, is not a commercially viable method. Thus, administration of the mutant bacteria is preferably carried out by immersing a fish in a suitable suspension of the avirulent bacteria for a period of time sufficient to effect an adequate immunization.") Fang et al. teach that the AHMA protein (although not in pure form) antigen can infer protection when injected intraperitoneally in the presence of FCA. Accordingly, one of ordinary skill in the art would not combine Fang et al. with Wolf-Watz et al. to derive an oral, live cell vaccine comprising recombinant AHMA protein or immunogenic recombinant AHMA fragments, or the recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis,* inactivated viruses, and bacterial antigens or killed bacteria.

Applicants's further assert that Wolf-Watz et al. and Fang et al. fail to disclose the use of immobilization antigen repeat 1 of *Ichthyophthirius multifiliis*. The Examiner states in the present Office Action: "In the instant case, Wolf-Watz et al teach vaccines comprising *Aeromonas hydrophila*. Wolf-Watz et al do not teach other antigens such as *Ichthyophythirius*. Wang et al teach vaccines comprising *Ichthyophythirius*. The teachings of Fang et al have been described previously." (Office Action, page 17, emphasis added). Applicants respectfully assert that it is improper to interject another reference in the Examiner's rationale for overcoming the Applicants's remarks in the previous Office Action when the reference was not used as the basis for the 103(a) rejection. Applicants respectfully submit, that it has overcome the present rejection for at least the reasons provided above.

Despite the improper reference citation, Applicants submit that while Wolf-Watz et al. also teach that the vaccine may comprise one or more antigens that are

pathogenic to fish in order to produce a vaccine that provides a broad spectrum protection against a range of fish pathogens (column 7, lines 62-66), Wolf-Watz et al. teach away from the present invention because the vaccine described by Wolf-Watz et al. comprises additional antigenic determinants that are genetically inserted into the avirulent strain using genetic techniques so that the live avirulent host cell expresses the added antigenic determinant. The present invention teaches oral vaccine compositions that comprise isolated recombinant proteins (i.e. recombinant protein AHMA and recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis)* optionally in combination with killed pathogens which are not expressed on the surface of live avirulent bacteria as a vehicle for immunological presentation.

Contrary to Wolf-Watz et al.'s teaching on the advantages of using live, whole cell vaccines over those based on isolated recombinant proteins, the present invention unexpectedly and surprisingly demonstrates that effective immunization against *A. hydrophila* can be achieved using isolated recombinant proteins (i.e. recombinant protein AHMA and recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius* multifiliis). As shown in Examples VI and Table 1 (pages 16-17 of the present Application), survival rates against *A. hydrophila* infection were significantly increased in fish immunized with an oral vaccine composition comprising the isolated recombinant AHMA protein in an orally suitable formulation.

Further, it would not be *prima facie* obvious to one of skill in the art to include both the isolated recombinant proteins and killed pathogens in an oral vaccine composition in the expectation that it will confer an effective broad spectrum of

protection against a range of fish pathogens since Wolf-Watz et al., describes the use of live vaccines to be superior to single antigens which are less effective than live whole cell components. As such, the use of Wolf-Watz et al. in combination with the "single component" major adhesion protein antigen of Fang et al. would not provide a basis for reasonable expectation of success by one of ordinary skill in the art to obtain an orally suitable oral vaccine capable of effecting immunization against *A. hydrophila* and other bacterial fish pathogens.

Accordingly, since Wolf-Watz et al. either alone or in combination with Fang et al. do not disclose each and every limitation found in Claims 1 and 48 and Claims which are dependent thereon, it is respectfully submitted that Wolf-Watz et al. in combination with Fang et al. do not render the rejected Claims 1-3, 5-6, 10, 15-16, 20-21, 27-36, 45, and 48 obvious to one of ordinary skill in the art. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

ITEM 11. Claims 11-13 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et al., Fang et al. for Claims 1-3, 5-6, 10, 15-16, 20-21, 27-26, 45, and 48 and further in view of Wang et al. (Fish Shellfish Immunol., November 2002, 13(5):337-50). This rejection is respectfully traversed.

Claims 11-13 are drawn to the oral vaccine of Claim 1 further comprising a recombinant protein comprising immobilization antigen repeat I of *Ichthyophthirius multifiliis.*

The teachings of Wolf-Watz et al. and Fang et al. are described above (see Items 6 and 10). Wang et al. disclose an injectable vaccine composition comprising the immobilization antigen repeat I of *Ichthyophthirius multifiliis* and Freund's Complete Adjuvant (FCA) and Freund's Incomplete Adjuvant. Wang et al. teach the use of administering the subunit vaccine with intraperitoneal injection and not as an orally suitable vaccine.

As discussed above, Wolf-Watz et al. teach away from the present claims as Wolf-Watz et al. discuss the advantages of live whole cell vaccines over vaccines based on killed pathogens or isolated recombinant proteins as single component antigens. Moreover, Wolf-Watz et al. clearly teach away from using single component antigens comprising AHMA set forth in SEQ ID Nos: 2, 4, or 8 and pathogenic antigens from other bacterial species, because Wolf-Watz et al. teaches genetic incorporation of antigens into the host avirulent strain and expressed by the live host strain.

Wolf-Watz et al, expressly states "[l]ive vaccines generally have the advantage over vaccines based on killed pathogens or bacterial components that they confer a higher degree of immunity as well as a more prolonged effect, and is more complete than when single components such as antigens are administered." (Wolf-Watz, Col. 2, lines 26-31). Hence, Wolf-Watz teaches away from using recombinant adhesin protein of *Aeromonas hydrophila* set forth in SEQ ID NOs: 2, 4, or 8 to form the orally suitable vaccine. Therefore, although, the Applicants agrees with the Examiner's statement: "Thus, Wolf-Watz et al contemplates the use of antigenic determinants or bacteria antigens." (Office Action, page 19), Wolf-Watz also states: "It would therefore be an

advantage to develop a live vaccine for administration to fish requiring a lower immunization dosage than existing fish vaccines based on killed pathogens or membrane components." (Wolf-Watz et al, col.2, lines 36-40). Applicants reiterate, that the present invention is not rendered obvious in light of the combination of Wolf-Watz et al. and Fang et al. further in view of Wang et al. because: 1) Fang et al. does not provide SEQ ID NOs: 2, 4, or 8 to form a recombinant adhesin protein of *Aeromonas hydrophila* to combine with Wolf-Watz, and 2) Wolf-Watz et al. teaches away from using recombinant membrane components such as recombinant adhesin protein of *Aeromonas hydrophila* in the absence of live cells to form the basis of antigenicity for the orally suitable vaccine. A reasonable reading of Wolf-Watz et al. would not fairly suggest that the Applicants' claimed invention would lead to a likelihood of success based on the fact that the present vaccine is not a live avirulent strain as recommended and taught in Wolf-Watz et al.

Therefore, it would not be *prima facie* obvious to combine Wolf-Watz et al. and Fang et al. with Wang et al. for reasons also discussed above in the expectation that the resulting oral vaccine composition having the additional isolated recombinant protein comprising the immobilization antigen repeat I of *Ichthyophthirius multifiliis* will confer an effective broad spectrum of protection against a range of fish pathogens.

Accordingly, since Wolf-Watz et al. either alone or in combination with Fang et al. and further in view of Wang et al. do not disclose each and every limitation found in Claims 1 and 48, and Claims 11-13, which are dependent thereon, it is respectfully submitted that Wolf-Watz et al. in combination with Fang et al. and further in view of Wang et al. do not

render the rejected Claims 1-3, 5-6, 10, 11-13, 15-16, 20-21, 27-26, 45, and 48, obvious to one of ordinary skill in the art. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

ITEM 12. Claims 22, 26, and 47 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Wolf-Watz et al., Fang et al., Wang et al. as set forth for Claims 1-3, 5-6, 10-13, 15-16, 20-21, 27-36, 45, and 48 and further in view of Morinigo et al. (*Bulleting of the European Associate of Fish Pathologists*, November 2, 2002, Vol. 22, No. 5, pp. 298-303). This rejection is respectfully traversed. It is presumed that that the Examiner inadvertently found claims 22, 26 and 47 to be dependent from Claims 1 and 48. Claims 22, 26 and 47 are dependent directly and indirectly from Claim 1.

Claims 22, 46, and 47 depend from the oral vaccine of Claim 1, and further comprise bacterial antigens or killed bacteria selected from the group consisting of *Shewanella putrefaciens, Pseudomonas fluorescens Vibrio alginolyticus* and *Photobacterium damselae*. The teachings of Wolf-Watz et al., Fang et al. and Wang et al. are described above in Items 6, 10, and 11.

Morinigo et al. discuss a divalent vaccine composition comprising formalized (i.e. killed and inactivated, see page 299) whole cells and extracellular products (ECPs) of virulent strains of *Vibrio alginolyticus* and *Photobacterium damselae*. As discussed above, Wolf-Watz et al. teach away from the present claims as Wolf-Watz et al. discuss the advantages of live whole cell vaccines having one or more bacterial pathogenic determinants expressed as a live avirulent bacterial vaccine over vaccines

based on killed pathogens or isolated protein components. Therefore, it would not be *prima facie* obvious at the time the invention was made to add the formalized *V. alginolyticus* and *P. damselae subsp. Piscicida* antigens as taught by Morinigo et al. to the vaccine composition of Wolf-Watz et al. even in combination with Fang et al. and Wang et al.

Accordingly, since Wolf-Watz et al. either alone or in combination with Fang et al., Wang et al. and further in view of Morinigo et al. do not disclose each and every limitation found in Claim 1, and Claims which are dependent thereon, it is respectfully submitted that Wolf-Watz et al. in combination with Fang et al. and Wang et al. and further in view of Morinigo et al. do not render the rejected Claims 22, 46, and 47 obvious to one of ordinary skill in the art. Applicants believe that these remarks overcome the rejection and respectfully request that it be withdrawn.

**CONCLUSION**

It is believed that all of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully requests that the Examiner reconsider and withdraw all presently outstanding rejections. It is believed that a full and complete response has been made to the outstanding Office Action and the present application is in condition for allowance. Thus, prompt and favorable consideration of this amendment is respectfully requested. If the Examiner believes that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (248) 641-1600.

Respectfully submitted,

Dated: May 16, 2007

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